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THE COMPOSITION AND ADULTERATION OF HONEY.

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THE COMPOSITION AND ADULTERATION OF HONEY

BY

EVELYN MARIE NIEDECKEN

A Thesis Submitted for the Degree of

MASTER OF ARTS

UNIVERSITY OF WISCONSIN

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INTRODUCTION

Honey is as old as history itself. It was known to the ancient Egyptians and the Hebrews, to the Greeks and the Romans, and to all the peoples of Asia. In Egypt, apiculture formed a considerable industry, but the Asiatic nations had no need of that since the wild bees supplied them with honey sufficient for their needs.

Since it was their chief source of sugar, honey formed an important article of food for the ancients. However, its uses were not confined to one. The ancient Egyptians used it in embalming. The Romans boiled it with wine and water and prepared their "mulsum," which came down to more modern times in slightly varied form as the old English "mead," and the "Piment" of Chaucer's day. Although it was forbidden as an offering in the Jewish Ritual, it was used in many heathen forms of worship. The libation, which the Greeks offered to the dead, consisted of honey and wine, and pure honey was offered as a sacrifice to the sun by the Peruvian aborigines. To the fabled serpent guardian of the

Acropolis a honey cake was offered monthly. Even now, on New Year's day, at Madagascar, the queen and her guests eat rice and honey.

PART I

We moderns are seldom, if ever, permitted to regale ourselves on the far-famed honey of Mount Hymettus, where the ancients dedicated a temple to Apollo, protector of the bees. We must content ourselves with honey less famed, yet, probably, no less delicious, provided the bee is allowed to produce honey as nature intended it should. Left free to follow its instincts, the bee goes out to gather the nectar from the flowers, returns to the hive, and stores the honey thus obtained in the cells which its co-workers have prepared. The nectar of the flowers, containing, according to Wilson,[1] mainly sucrose and levulose was thought by Kraut[2] and Röders[3] to undergo a change in the bee's body, a theory later confirmed by von Planta and Erlenmeyer[4] who succeeded in isolating from the bee's body a ferment having power to invert cane sugar, and to change starch to dextrose. It

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[1] Pharm. Jour. Trans., (3) 9:225.
[2] Zeitschr. f. Chem. und Pharm., 6:359.
[3] Arch. d. Pharm., (2) 116:29.

would seem probable that some of this ferment goes over into the honey itself. After the honey has been stored in the cells, and before the latter are sealed, the bee introduces into it a slight quantity of formic acid, whose source is the sting of the bee, and whose purpose Müllenhoff[1] discovered to be that of a preservative. He arrived at this conclusion after noting that uncapped honey contained no formic acid, and fermented readily, while a small quantity of formic acid added to this same honey prevented any fermentation.

Although the honeys thus gathered vary according to their age, and with the season and the climate, their general composition remains the same. Invert sugar forms the chief constituent, levulose being, perhaps, slightly in excess. That cane sugar is a normal constituent of honey was for a long time a matter of dispute. Kraut,[2] in 1863, stated absolutely that honey contained no cane sugar, although, in 1849, Soubeiran[3] had mentioned the presence in honey of a sugar capable of inversion, along with two other sugars, one of which was grape sugar, while the other did not crystallize and was twice as strongly levo rotatory

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[1] Rep. Anal. Chem., 84:331.
[2] Zeitschr. f. Chem. and Pharm., 6:359.
[3] Compt. Rend., 28:744, 745.

as inverted cane sugar. Even as late as 1884 Elsner[1] stated that pure honeys contained only invert sugar. Barth[2] in the following year reported the presence of cane sugar in some undoubtedly pure honeys, and, since that time, a small though varying percentage of cane sugar has been considered a normal constituent of honey. Whether this varying factor is due to the fact that the bee is able to invert only a limited amount of cane sugar in the nectar of the flowers, the amount varying with the individual or with the time of the year, is still a matter of conjecture. Honey contains a considerable percentage of water, and a very small ash content. In addition there are slight amounts of nitrogenous substances, and, sometimes, traces of wax and pollen. Planta-Richenau[3] added to the constituents already given albumen, peptones, fat, lecithin, salts, ethereal oils, and a ferment capable of inverting cane sugar and of converting albumins to peptones.

Concerning the nature of this ferment some work was done by Axenfeld[4] in 1903. He claimed it to be invertin

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[1] Rep. Anal. Chem., 84:186.
[2] Pharm. Ctr.-H., 26:87 to 93.
[3] Ibid., 21:202, 203.
[4] Ctr. Bl. f. Physiol., 17:268, 269.

having its source in the body of the bee. Langer.[1] in the same year, declared that the ferment had two sources, namely, the saliva of the bee, and the nectar of the flowers. In demonstration of this statement he claimed to have isolated the ferment from buckwheat honey, and, by inoculating a squirrel therewith, to have obtained a serum which would react with buckwheat honey, producing a precipitate. He stated his belief in a possibility of obtaining specific serums for the various varieties of flower honey, these to form an unfailing test for the purity and character of any honey in question. Marpmann[2] divided honey ferments into three kinds; the first, an inverting ferment, the second, an alcohol producer, and the third, proteolytic in character; that is, possessing the power to convert albumins into soluble peptones. These ferments are present only in fresh honey, never in boiled honey. Furthermore, Marpmann claimed that the presence of these ferments could be determined by certain color reactions. Though their value as a proof of purity may well be questioned, the presence of these ferments seems to be undoubted.

The albuminous constituent of honey, already touched upon in the discussion of ferments, was brought to notice by

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[1] Schweiz. Woch. f. Pharm., 41:17, 18.
[2] Pharm. Zeitung. 1904, p. 1010.

Erlenmeyer and von Planta[1] in 1874. Upon boiling, filtering, and evaporating a honey solution, they obtained an alcoholic solution of the residue which proved to be albuminous in character. At the same time they noted that the nectar of flowers contained a similar nitrogenous constituent, although they did not consider it an albumen. The subject was taken up again in 1902, when Bräutigam [2] isolated the same albuminous constituent by means of acetic acid and a saturated solution of NaCl. In this investigator's opinion the albuminous constituent partook of the character of a peptone and had its source in the saliva of the bee. He proved his statement by the fact that honey produced entirely by cane sugar feeding contained this peptone. On the other hand, purely artificial honey contained none of it. In flower honeys of known purity, he found both peptone and albumin.

Several minor constituents of pure honey remain to be mentioned. First of these is phosphoric acid which Hehner [3] claimed to exist in pure honeys to the extent of 0.013 to 0.035 percent. Scheitz [4] made mention of some aldehydes which he believed existed in all pure honeys, giving them flavor and odor. Of the action of honey under polarized

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[1] N. Rep. Pharm., 23:610.
[2] Pharm. Zeitung., 47:109.
[3] Analyst, 1885, p. 217.
[4] Archiv. d. Pharm. (3) 1:423.

light, upon analysis, and upon fermentation, more will be said at a later time. Very recently, in 1905, Hilger called attention to the invariable malic acid content of honey.

A dextrinous substance in pure honey was not thought possible until 1885, when Barth [1] stated his belief that all honey contained it in greater or less degree. Up to that time dextrin had been considered an indication of adulteration. In the previous year Haenle [2] had published his work on coniferous honeys which differed from ordinary honeys in that they rotated the plane of polarization to the right, and in that they contained marked amounts of a dextrin-like substance. Barth's belief that even nectar honeys contained some dextrin-like substance was later corroborated by Mader [3] in 1890. He believed that the honey dextrin had its source in the nectar of the flowers, or else formed some intermediate stage in sugar production. Just what is the nature of this honey dextrin, which is present in the pine tree honeys, is still unsettled. Amthor and Stern,[4] in 1889, isolated it from the dextro rotatory honeys and determined some of its properties. They believed it directly un-

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[1] Pharm. Ctr. H. 26:87 to 93.
[2] Pharm. Zeitung. , 1884. P. 787.
[3] A. H. 10:399 to 444.
[4] Zeitschr. Ang. Chem., 1889. P. 64.

fermentable but capable of inversion into dextrose. The glucosazon derived from it coincided with the one derived from pure dextrin. During the same year von Raumer [1] agreed that this dextrin-like substance was unfermentable, in fact, he isolated it by means of fermenting a 10 percent solution of honey and precipitating the dextrin with alcohol. Upon inversion he found the substance to be fermentable. He believed it similar to gallisin in its optical activity, but dissimilar in its reducing power. In 1891 Beckmann and Hilger [2] stated their belief that this honey dextrin belonged to the bioses. They discovered that it was soluble in methyl alcohol. At a later time von Raumer [3] slightly revised his former statement. He still affirmed the presence of a dextro-rotatory substance in all honeys; since all honeys become dextro-rotatory upon fermentation with beer yeast, a process which destroys the invert sugars, acting upon the dextrose even more rapidly than upon the levulose. Unless some such dextro-rotatory substance was assumed, the dextro-rotation of the fermented honey could not be explained. Furthermore, if honey were made up of pure invert sugar it would rotate considerably to the left, when, as a matter of

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[1] Zeitschr. Ang. Chem., 1889. P. 64.
[2] Ibid., 1891. P. 571.
[3] Zeitschr. Anal. Chem., 33:397 to 408.

fact, in spite of the excess of levulose over dextrose in pure honey, its rotation was usually very slightly levo and sometimes dextro. Thus a dextro-rotatory substance, counteracting the natural levo-rotation, had to be assumed. He believed this substance to be intermediate between dextrin and sugar. Beckmann,[1] in 1896, precipitated various dextrins by means of barium hydroxide and discovered that the amount of precipitation varied with the molecular weight of the dextrin. Since honey dextrin precipitated but slight amounts as compared with starch dextrin, he concluded its nature to be that of an achroo dextrin of low molecular weight. At the same time, he noted that nectar honeys formed a flocculent precipitate with methyl alcohol, while pine-tree honeys formed scarcely any. On the other hand, ethyl alcohol precipitated the dextrin from the pine-tree honeys quite completely. During the same year, Künnemann and Hilger [2] isolated honey dextrin and concluded from its $[\alpha]_d$ and its reducing power that it partook of the nature of an achroodextrin with a slight admixture of maltose. Sometime later, in 1903, Haenle and Scholz [3] isolated the dextrin from pine-tree honey by means of fermentation with wine yeast and found it to contain 6.3 percent of directly re-

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[1] Zeitschr. Anal. Chem., 1896. P. 263.
[2] Forschb. ü. Lebensm. etc., 3:329, 330.
[3] Zeitschr. Unters. Nahr.-Genussm., 1903. Pp. 1027 -1031.

ducing substance, 9.28 percent of a substance which became reducing after an inversion of one-half hour, and 26.44 percent of a substance which reduced after an inversion of three hours. Furthermore, they stated that the pure pine-tree honey rotated the plane of polarization +21°, while after fermentation, which destroyed both dextrose and levulose, the dextro-rotation had increased to +50°. The increase could have been accomplished only by an unfermentable dextro-rotary constituent. In the following year Hilger[1] isolated the dextrin by means of a mixture of ethyl and methyl alcohols. He stated the nature of this substance as akin to an achroo-dextrin, an intermediate between starch and sugar. Furthermore, he claimed that every coniferous honey possessed a characteristic dextrin of constant specific rotation. All of these honey dextrins reduced Fehling's solution, becoming hydrolyzed by the alkali. They did not form osazones, and were with difficulty, sometimes not at all, fermented by strong organisms.

These abnormal honeys, already spoken of as pine tree honeys, or coniferous honeys, were first examined and their existence affirmed by Haenle [2] in 1884. Hitherto,

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[1] Zeitschr. Unters. Nahr.-Genussm., 1904. Pp. 110 to 126
[2] Pharm. Zeitung., 1884. P. 787.

it had been supposed that pure honeys invariably rotated the plane of polarization to the left and were free from dextrinous substance. Haenle stated positively that pure dextrorotatory, dextrin-containing honeys existed, and his statement was confirmed in the following year by Klinger[1] and also by Amthor[2] who supposed this dextro-rotatory, dextrin-like substance, which he had isolated by means of alcohol, to be derived from the pine tree exudations or honey dew. These honeys contained traces of chlorides and sulphates and differed from nectar honeys mainly in their dextro-rotation, their large content of a dextrin-like substance, and their dark color, as compared with most nectar honeys.

In 1894, von Raumer published some analyses of pine tree honeys, as follows:-

Sugars, Direct, - -	68 to 88 %.
Sugars, Invert, - -	77 to 90 %.
Rotation, Direct, - -	(-0.4° to + 4.7°)
Rotation, Invert, - -	(-1.0° to + 2.4°)
Rotation, after Fermentation, - - - -	(+3.8° to + 7.66°).

The honey dew from which, in dry seasons especially, the bees manufacture the pine tree honeys, is an exudation

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[1] Rep. Anal. Chem., 1885. 5:166 to 168.
[2] Ibid., 1885. Pp. 163 to 166.

from the pine trees. Various analyses of this honey dew have been given,- the first by Wiley[1] in 1891. His results were as follows:

Water,	54.40 %,	(Invert sugars,	17.44 %.
Solids,	45.59 %,	(Cane sugar,	8.16 %.

leaving 19.99 percent of solids unaccounted for. From the disparity in the polarization of the honey dew, and the calculated polarization, due to the invert sugars, he realized the presence of a strongly dextro-rotatory constituent, which he concluded to be arabinose with $[\alpha]_d = 105°$. In 1894, von Raumer[2] recalled some older analyses of honey dew. The earliest one was given by Dobereiner: uncrystallizable, fermentable sugars, mucous, and traces of albumen and of a characteristic acid. Later Unger reported:

Unger:	Dextrose, - - - -	25.00 %.
	Gum, - - - -	8 to 20.00 %,
	Insoluble matter - -	0.75 %.
	Mannite, salts, etcetera,	53.00 %.
Boussingault:	Cane sugar, -	48 to 58.00 %.
	Invert sugar -	24 to 28.00 %.
	Dextrin, - -	19 to 22.00 %.

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[1] Am. Chem. Jour., 13:24.
[2] Zeitschr. Anal. Chem., 33:397 to 408.

Von Raumer added an analysis of his own:

Sugar direct, 16.00 to 17.00 %,

Sugar invert, 28.50 to 28.60 %,

Dextrin, - - - 39.40 %, after fermentation with compressed yeast.

Ash - - - - 3.02 %,

Nitrogen - - - 3.17 % (calculated as albumins.)

Rotation, direct - - - + 22 to 23°.

Rotation, invert - - - + 20 to 21°

Rotation after fermentation - + 66.5 °

The large difference between the sugars before and after inversion indicated a large cane sugar content. After fermentation he calculated back from the alcohol produced and found a larger percentage of sugar indicated than was shown by reduction. He concluded the presence of some intermediate fermentable, non-reducing substance in the honey dew. Likewise, he called attention to the large percentage of ash, as compared with normal honey. The high dextro-rotation after inversion he considered as another indication of the presence of some highly dextro-rotatory substance. He stated his belief that the dextrins of the honey dew were changed in the body of the bee so as to become more easily dialyzed.

There are other kinds of honey dew besides that obtained from the pine tree. In 1877, Hoffmann[1] noticed a sticky sweet substance upon the leaves of a house plant, the Camellia Japonica. This honey dew consisted mainly of gummy material. It was not due to the presence of the aphis, since no trace of insects could be found. Bornier,[2] in 1896, also stated his belief in a honey dew not due to the presence of the aphis. It resembled the nectar of flowers more than ordinary honey dew,- being composed of levulose, dextrin, and gum, but no cane sugar. Physically, it was odorless, sweet, and hygroscopic. His theory was that it appeared at night when great changes of temperature occurred. An analysis given by Mr. Wiley,[3] in 1893, gave the composition of a honey having its source in honey dew appearing as the result of the presence of the aphis.

Invert sugar, - - -	71.50 %,
Cane sugar, - - -	6.04 %,
Ash, - - - - -	0.62 %,
Other solids, - - -	21.76 %.

The solids unaccounted for were thought, by this investigator, to be arabinose $[\alpha]_d = 105^\circ$, possibly. This honey

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[1] Jahresb. Fortschr. Chem., 1877. P. 928.
[2] Compt. Rend., New Eng. Dr., 1896. P. 666.
[3] Jour. Amer. Chem. Soc., 1893. P. 350.

dew honey contained a low percentage of invert sugar, a high percentage of ash, and was dextro-rotatory. In 1900, Maquenne[1] reported on a honey dew present on the Camellia Japonica. It appeared in dry seasons, always accompanied by the aphis. Crystals of dulcite formed in the concentrated solution, while the mother liquor contained invert sugars. He believed this honey dew to be the result of the perforation of the plant cells by the sting of the aphis.

There are a number of other honeys made by the bee, which are rare and, therefore, do not enter to any extent into commerce. One of these is eucalyptus honey, a sample of which was found by a French traveller and sent to Paris for analysis. It was found to contain:[2]

Sugar, - - - -	61.10 %,
Water, - - - -	21.58 %,
Ash, - - - -	0.18 %,
Eucalyptol, Eucalyptene, Terpene, Cymol, et cetera,	17.10 %.

It possessed the taste and odor of eucalyptus oil; was deep orange in color, and was said to have medicinal properties. Maquenne,[3] in 1890, reported a eucalyptus honey, made by

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[1] Bull. Soc. Chim. (3) 21:1082, 1083. Paris.
[2] Pharm. Ctr.-H., 28:444.
[3] Ann. Chim. Phys., (6), 17:495 to 500.

black bees, and stored up in immense hives in the summits of the eucalyptus trees. The honey was thick and aromatic; containing invert sugar, traces of gum, and a small amount of aromatic substance. Again, in 1891, Passmore,[1] mentioned a eucalyptus honey which was dark, syrupy, tending to crystallize and faintly aromatic. It contained dextrose, levulose small amounts of galactose, but no cane sugar. It was obtained by the bees from a substance which was white, semi-crystalline, sweet, and aromatic, soluble in water and absolute alcohol. It reduced strongly, although the sugar constituent was non-reducing before inversion and reducing after inversion. He concluded that it was similar to melitriose, a condensation product of galactose, dextrose, and levulose.

Another so-called medicinal honey was described in 1879 by Villiers;[2] it was an Ethiopian honey, produced by a mosquito-like insect, and stored up underground without any wax. The natives called it "Tazma" and used it as a cure for throat trouble. Its composition was:

Water - - -	25.5 %,	Dextrin - -	27.9 %,
Fermentable sugars, Levulose and Dextrose in excess	32.0 %,	Mannite - -	3.0 %,
		Ash - - -	2.5 %.

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[1] Proceed. Amer. Pharm. Assoc., 1891. P. 443.
[2] Compt. Rend., 88:292.

It contained, also, a slight bitter principle. There was no cane sugar present, thus differing from honey and honey dew.

In 1882, Franchimont[1] described a honey from Sumatra, produced by the Apis Indica. It contained:

Water - - -	26.60 %,
Ash - - -	0.23 %

Wax, pollen, maize,-traces.

The remainder consisted of invert sugars in the ratio of:

Levulose : Dextrose = 5.92 : 7.41

There was neither cane sugar nor dextrin present.

The Mexican Honey Ant manufactures a honey described by Wetherill,[2] in 1853. It was composed mainly of levulose with some cane sugar. A characteristic acid, resembling formic acid was present. The honey was dark in color and sweet in taste.

In 1857, Karsten[3] mentioned a honey made by a species of wasp. The honey resembled that made by the bee, and contained large crystals of cane sugar, which was at that time considered an impurity if present in honey.

There remain for mention the poisonous honeys, which in ancient times laid low Xenophon's ten thousand Greeks who had

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[1] Rec. Trav. Chim., 1:223. Pays-Bas..
[2] Chem. Gaz., 1853. Pp. 72 to 74.
[3] J. Prakt. Chem., 71:315.

partaken of it too freely. Pliny mentioned it at a later time, ascribing its poisonous properties to the Azalea Pontica. It resembled pure honey in physical appearance and, as Thresh[1] stated in 1887 was derived from the Azalea Pontica, which containsa poisonous principle, hence, the toxic properties of the honey.

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[1] Pharm. Journ. Trans. (3); 18:397.

PART II

The adulteration of honey has been practised for many years, the cruder methods of earlier years giving place to the more skillful falsification necessary to meet greater knowledge of the true composition of this food, until the chemist often finds it difficult to prove an adulteration.

In literature, the first mention of adulterated honey seems to have been made by Lassaigne,[1] in 1845. French honeys, he stated, were largely adulterated with glucose. Later, in 1870, Hager[2] described a so-called Swiss Table Honey, consisting of a mixture of honey and glucose. A somewhat similar honey was described in 1886 by Amthor,[3] which consisted of a mixture of honey and inverted starch paste, and which sold under the name of Dr. Eschmann's Schweizer Alpenhonig. These honeys usually resembled pine honey in physical appearance, except for the fact that they would not

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[1] J. Chem. Med., 1844. Pp. 395 to 398.
[2] Pharm. Ctr.-H. 11:241.
[3] Schweiz. Wohschr. Pharm., 1886. P. 202.

crystallize upon standing.

Up to 1884 it was thought that glucose might be profitably added indirectly to honey by feeding. In fact, in 1863, both Kraut[1] and Röders[2] mentioned such an adulteration. The former described the honey so derived as hard, yellowish, not very sweet, and containing only dextrose. From this fact he concluded that the bee stored up dextrose without any change. Röders also examined such honeys, finding them to contain dextrose together with another dextro-rotatory, non-reducing substance, not dextrin. Later, in 1869, a statement appeared in the Schweizer Wochenschrift[3] to the effect that bees had been fed on glucose with a resulting honey resembling pure honey. Elsner.[4] in 1884, was the first to mention that glucose could not with profit be fed to bees, since it caused disease. He found that the bees refused glucose, but would feed upon a mixture of glucose and honey. The resulting honey was dark, thin, and slightly dextro-rotatory. According to Beckmann,[5] in 1896, such a honey produced only cloudiness with methyl alcohol as well as with methyl alcohol and $Ba(OH)_2$ while with iodine it gave

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[1] Zeitschr. Chem. Pharm., 6:359.
[2] Arch. d. Pharm., (2) 116:29.
[3] Schweizer. Wochschr., Pharm., 1869.
Pharm. Ctr.-H., 10:79.
[4] Rep. Anal. Chem., 1884. P. 186.
[5] Zeitschr. Anal. Chem., 1896. P. 263.
Forschb. u Lebensm.. 3:329, 330.

no color reaction, thus resembling pure honey in these respects.

Admixture of cane sugar has been practised to some extent, and much of this adulteration is accomplished through feeding the bees with cane sugar syrup. Barth[1], in 1885, mentioned this as injurious to the bees; however, it is frequently resorted to, more especially during the winter months when other food is lacking. Hehner[2] considered that such honeys contained no cane sugar though his statement was disproved later by Lippmann's data on such honeys.[3] The honeys described had resulted when the bees resorted to sugar refineries, and the cane sugar-content sometimes became as high as 16.38 percent. Hefelmann[4] and Utescher[4] carried on a lengthy polemical discussion as to the proper classification of such honeys. The former argued that, inasmuch as the bee-keeper could not control his bees, a cane sugar content having its origin in the sugar syrups of the refineries could not be considered an adulteration. The latter affirmed just as strongly that such a product was not "Honey" since it had an origin other than the nectar of flowers.

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[1] Pharm. Ctr.-H., 26:87 to 93.
[2] Analyst, 1884. P. 64.
[3] Schweiz. Wochschr. Pharm., 1889. P. 133.
[4] Pharm. Ctr.-H., 1894. Pp. 36, 37, 39, 481, 513, 552.

According to Hefelmann,[1] when the bees are fed on cane sugar syrup alone, they are able to invert only two-thirds of the cane sugar. Some interesting experiments in cane sugar feeding were carried on in 1899 by Haenle.[2] A 33 % solution of cane sugar, rotating +96° and non-reducing, was fed in three portions. The first portion was flavored with oil of roses, the second, with anise, after inversion with wine yeast, while the third portion was unflavored. The resulting honey, in each case, was odorless, sweet, brown in color, and rotated - 3°. The first honey contained slight traces of dextrin. A honey which had its source partly in nectar of flowers, and partly in the syrups of a canning factory, was brown, sweet, waxy in taste, contained traces of dextrin, and rotated -12° to -15°. von Raumer,[3] in 1902, experimented along this same line and concluded from his results: 1st, that bees invert all but a small percentage of cane sugar; 2nd, that bees change fermentable, non-reducing sugars; 3rd, that bees do not thrive on dextrinous food. He believed that the honey dextrins differed from starch dextrins, and that the dextrins were changed by the yeast enzyme, first to sugar and later to alcohol.

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[1] Pharm. Ctr.B.-H., 35:481.
[2] Pharm. Zeitung., 1899. P. 742.
[3] Zeitschr. Anal. Chem., 1902. Pp. 333 to 350.

The most skillful adulteration of honey consists in the manufacture of an entirely artificial honey by a new process of inverting cane sugar. The operation of inversion is accomplished by carbonic acid gas under high pressure. After the invert sugar has been obtained, the pressure is released and the carbon dioxide is given off. No tell-tale impurities are left behind in process of manufacture, and the invert sugars are not to be distinguished from those normally present in honey. After the addition of a few percent of cane sugar, some artificial coloring and flavor, with a few other additions to produce the proper amount of ash, the product fairly defies detection, to say nothing of proof. As early as 1887[1] a patent was published by which honey could be made from 35 parts dextrose, 40 parts levulose, cane sugar sufficient to give to a 10 % solution a rotation of 1° (Soleil - Ventzke) and essences to supply the odor and taste of honey. The essence may possibly have been butyric ether. The whole was heated to homogeneity, after which a little honey was added to give it the desired granular appearance. In 1891, Weigle[2] reported an artificial honey consisting of invert sugar, water, mineral matter, a little free acid, and essences to supply the odor and taste

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[1] Pharm. Ctr.-H., 28:692.
[2] Chem. Zeitung, 15:1053.

of honey. Also, Claasen,[1] in 1898, mentioned an adulteration composed of invert sugar and pure honey. Two very recent commercial products were mentioned by Beythien,[2] in 1905, sold under the name of "Honamin," and "Fruktin." The former contained 20.03 % of water, 54.54 % of invert sugars, 23.46 % cane sugar with slight amounts of tartaric and formic acids. This product upon being boiled contained 69 % of invert sugar, 17 % water. 14 % cane sugar, and artificial coloring.

One artificial honey, so extraordinary as to meet special notice was reported in 1887.[3] It was a Dutch product and consisted of a mixture of invert sugar, cane sugar, dextrin, oleomargarine, and ash constituents.

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[1] Chem. Tech. Rep., 1898. P. 510.
[2] Zeitschr. Unters. Nahr.-Genussm. 1905.
[3] Pharm. Ctr.-H., 28:66.

PART III

During the earlier part of the past century, the published work on honey was limited mainly to methods of purification. To be sure, its action under the blow pipe, with oxalic acid, with copper compounds, and with borax was noted but all of these earlier researches were scattered, unsystematic, and unscientific. At present the analysis of honey is generally confined either to the analysis of a sample of known purity with the object of increasing our knowledge of the composition of pure honeys, or to the analysis of samples of unknown origin, for the purpose of detecting adulterations. A brief discussion of some of the methods used in such analyses follows.

The specific gravity of honey which in 1834 was considered by Völter[1] a criterion of purity, 1.430 to 1.425 being considered the limits of a pure honey, was proved valueless as a test by Schumacher - Kopp,[2] in 1881, since glucose

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[1] Chem. Ctr.-Bl., 5:432.
[2] Chem. Zeitung., 1881. P. 646.

honeys varied between 1.425 and 1.445 while pure honeys varied between 1.39 and 1.45. Indeed, as Levy,[1] in 1902, pointed out, young honeys sometimes run as low as 1.072 to 1.095. Specific gravity is, however, found of use in computation of the percentage solids in a honey.

The method of making moisture determinations in honey have undergone considerable revision. In 1885 Wiley and Broadbent [2] devised a method of dehydration according to which the honeys were dissolved in alcohol, and dried over sand at 98.5° C., for six hours. Mansfeld[3] in 1891 suggested drying over sand, at a temperature below 100° C., until a constant weight was attained. He gave 20 percent water as a maximum. Villaret[4] in 1893 favored drying in vacuo at 80° C., and subsequently in an oven at a higher temperature. He considered 23.34 percent of moisture an average. Hoitsema[5] in 1899 suggested drying in vacuo for a week or until a constant weight was attained. His object was to obviate the use of heat and the consequent danger of decomposition of the levulose. Shutt and Charron[6] in 1903 advo-

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[1] Pharm. Zeitung., 1902. P. 227.
[2] Chem. News, 52:280.
[3] Zeitschr. Allg. Österr. Apoth.-Ver., 1891. P. 339.
[4] Pharm. Zeitschr. Russ., 1893. Pp. 55 to 71.
[5] Zeitschr. Anal. Chem., 1899. Pp. 439 to 441.
[6] Chem. News, 1903. Pp. 195, 196, 210 to 212.

cated the use of diminished pressure at 60° to 70° for a period of several days. Whenever decomposition of the levulose was avoided, the method became impracticable by reason of its length. Therefore, the method now in general use consists in a determination of the specific gravity of a solution of definite strength and computation of the percentage solids from a table. The moisture will be the difference between one hundred percent and the percentage solids.

In analysis of honey for adulteration, the percentage of ash proves of value only when used in conjunction with other data. As a rule, the figures are low: according to Dott,[1] 0.2 percent, according to Allen,[2] 0.1 to 0.25 percent, and, according to Racine,[3] 0.24 to 0.3 percent. A lower figure indicates cane sugar or invert sugar, while a much higher figure indicates glucose. In 1890, Wiley and Edson[4] showed that carriers were more often a hindrance than a help in ashing, since they might interfere with further qualitative analysis of the ash. The process of ashing honeys is identical with that followed in syrups and sugars of all kinds. The honey is evaporated to dryness, charred and leached. The residue is ashed, the leachings are added, and

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[1] Chem. and Dr., 1898. P. 463.
[2] Ibid.
[3] Zeitschr. öffent. Chem., 1902. Pp. 281 to 286.
[4] Chem. News, 62:242.

after a second evaporation the whole is heated to a low red heat.

In 1876, Menière[1] found some French honeys which had been colored with roasted, powdered bread, in imitation of the dark Brittany honeys. In modern times small quantities of yellow coal tar dyes are added to accomplish the same result. To determine the presence of these dyes, the Arata wool test is used. According to Ley[2] and Bomer[3] natural honeys do not dye wool and do not turn red with HCl unless heat is applied. Yellow coal tar dyes, on the contrary, dye wool, and turn red with HCl in the cold.

Pollen and wax are sometimes estimated, according to Hoitsema,[4] by collecting on a filter, drying, and weighing. Since they vary in quantity and quality, their presence is no criterion of the purity of a honey.

Optical activity, as such, can not be regarded as a test for purity. To be sure, nearly all honeys are levo-rotatory, and up to the time of Haenle's discovery of dextro-rotatory honeys any dextro-rotation had been considered an indication of glucose. Lenz's test,[5] setting a smaller rotation than - 6° 30 ' for a honey solution of definite

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[1] Rep. d. Pharm., 1876. P. 610.
[2] Zeitschr. Unters. Nahr.-Genussm., 4:828, 829.
[3] Ibid., 8:364 to 366.
[4] Zeitschr. Anal. Chem., 1899. Pp. 439 to 441.
[5] Ibid., 1885. Part 4, p. 135.

strength as a sign of adulteration, was, by Haenle's work, proven fallacious. However, the polarization of a honey continues to be an important factor in a more indirect way, since it is used in Clerget's formula for cane sugar content.

From time to time various reagents have been suggested which were expected to give characteristic reactions with pure honeys. Lead subacetate was for a long time used as a test for purity. It was said to produce cloudiness or opalescence in pure honey and a heavy precipitate with adulterated honey. This test was pointed out as fallible by Utescher[1] in 1895, since he found pure honeys which gave a heavy precipitate while some adulterated honeys gave only an opalescence. The precipitation was due to the presence of the free organic acids, such as malic acid, and of albuminous matter, and, naturally, varied in amounts. The tannic acid and ammonia test was also criticized by Lenz, inasmuch as the color reactions which distinguished pure honeys from cane sugar honeys became useless in the case of dark honeys. Ley,[2] in 1893, published a method of detecting more than 20 percent of invert sugar, 10 percent of cane sugar, glucose, and molasses, by the use of his special reagent. It was de-

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[1] Apoth. Zeitung., 1895. 10:278, 279; 285 to 287.
[2] Pharm. Zeitung., 48:603, 604.

signed to give certain characteristic color reactions and a fluorescence in pure honeys, due to the presence of volatile substances present. Marpmann[1] devised a special apparatus in connection with which he uses several reagents. The amount of precipitate formed the criterion of purity. Ihl[2] in 1890 invented some color tests with phenols, depending upon the aromatic compounds in pure honey. Phloroglucin with HCl gave a pale red; resorcin with HCl gave a bright red; anilin sulphate with HCl gave a yellow color. The barium and silver nitrate tests are no longer considered of any value, since the glucose used for adulteration is produced free from sulphates and chlorides. A reagent suggested by Hager[3] in 1885 was intended to detect glucose by the color and the precipitate produced. Beckmann[4] in 1896 claimed to be able to establish glucose and dextrin adulteration by means of the color reaction with iodine solution. All of these special reagents, depending in most cases on the presence or absence of variable substances, have enjoyed only passing favor. In present day analyses the percentages of ash, solids, reducing sugars, and cane sugar are preferred to the more empirical reactions with these special reagents.

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[1] Schweiz. Wochschr. Pharm., 40:590, 591.
[2] Chem. Zeitung., 1890. P. 3.
[3] Pharm. Ctr.-H., p. 327.
[4] Zeitschr. Anal. Chem., 1896. Pp. 263 to 284.

Microscopy of honey has also been suggested from time to time, as a method of analysis. Hager[1] favored its use in detection of starch and in the identification of characteristic pollen particles and of wax. Since the last mentioned substances may be readily added to an artificial honey, their presence can be no proof of purity. Another attempt to establish a microscopical analysis of honeys was made by Pfister, but was disqualified for similar reasons.

When glucose adulteration was first mentioned by Lassaigne,[2] in 1845, its detection was a simple matter because it invariably contained $CaSO_4$ as an impurity which was detected by means of barium chloride and ammonium oxalate. When glucose came to be more carefully manufactured this method of detection became useless.

Still another method, depending upon the dextrin content of glucose, was proposed in 1880 by Planta Reichenau.[3] This consisted in the addition of alcohol to the honey in question. Opalescence indicated a pure honey, while a heavy precipitate indicated glucose. Only recently, in 1902, Levy[4] pointed out that mere opalescence was sometimes produced in

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[1] P. C. H., 23:54, 55.
Forschb. Leb. Hyg., 1895, I:29.
[2] Journ. Chem. Med., 1844. Pp. 395 to 398.
[3] Pharm. Ctr.-H., 21:202, 203.
[4] Pharm. Ctr.-H. 26:87 to 93.

artificial honeys, probably in cases where dextrin-free glucose was used. Planta Reichenau's method was modified by Klinger.[1] He dissolved the alcoholic precipitate in water and determined the polarization of the solution. Inactivity indicated pure honey, while dextro-rotation indicated glucose. To this subject Barth[2] added the statement that in the case of pure honey the precipitate is flocculent, while in the case of glucose it is sticky and amorphous. Hager[3] in the same year noted that alcohol added carefully to glucose honey formed a milky contact layer, while in pure honey no contact layer is formed. Beckmann[4] in 1896 discovered that methyl alcohol formed a heavy precipitate with glucose and a flocculent one with normal honey, with pine tree honey and grape sugar honey, while ethyl alcohol formed a heavy precipitate with pine tree honeys or grape sugar honeys. Moreover, he used $Ba(OH)_2$ and methyl alcohol to determine quantitatively, by the amount of precipitate formed, the amount of glucose used.

Another method devised by Planta Reichenau[5] depended upon the fact that the reducing sugars in pure honeys, which

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[1] Rep. Anal. Chem., 1885. P. 166.
[2] Pharm. Ctr.-H., 26:87 to 93.
[3] Chem. Ctr.-Bl., 1885. P. 764.
[4] Zeitschr. Anal. Chem., 1896. Pp. 263 to 284.
[5] Pharm. Ctr.-H., Pp. 202, 203.

were 63 to 71 percent before inversion, increased by about 8.33 percent upon inversion with H_2SO_4, due to the cane sugar content. The reducing sugars in glucose honeys, on the other hand, were originally only about 37 percent, but increased about 44 percent after inversion according to his method.

The method of analysis depending upon the fermentation of honeys was introduced by Sieben in 1884.[1] His data led him to affirm that pure honey after fermentation with yeast became inactive while those containing glucose became dextro-rotatory, due to the presence of unfermentable dextrin. This statement was confirmed in the same year by Hehner.[2] In the following year Klinger[3] stated that pine tree honeys, originally dextro-rotatory, became inactive or slightly levo-rotatory. The method was, however, in his opinion impracticable on account of its length. Barth[4] stated that he found some pure honeys which became dextro-rotatory after fermentation, though this might have been the result of incomplete fermentation. He believed that a rotation of more than +12° after fermentation indicated glucose. Sieben's

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[1] Zeitschr. Anal. Chem., 1885. P. 136.
[2] Analyst, 1884. P. 64..
[3] Rep. Anal. Chem., 5:166 to 168.
[4] Pharm. Ctr.-H., 26:87 to 93.

method, briefly, consisted in fermentation with compressed yeast at room temperature for a period of forty-eight hours. Subsequent inversion did not change the activity of pure honey, but glucose honeys became slightly less dextro-rotatory, due to a change from dextrin to dextrose. Kayser[1] added to this method that more than +1° rotation after inversion indicated admixture of glucose, also that when the dextrin converted by inversion to dextrose was determined by Allihn's method more than 1 percent dextrose indicated admixture of glucose. In 1889, Gastine[2] claimed that honey would not ferment completely except upon admixture of a prepared yeast food, for the yeast, containing nitrogenous matter. von Raumer-Erlangen[3]-in the following year disputed the need of such a food. The degree of fermentation, he declared, depended upon the kind of yeast used. Most complete fermentation could be obtained by the use of compressed yeast, while beer yeast was weaker and wine yeast, weakest of all. Mader[4] in the same year discarded Sieben's method, as such, but believed that fermentation might be profitably applied, in a case of pronounced dextro-rotation, to distinguish between glucose and dextrose. In such a case fermenta-

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[1] Zeitschr. Anal. Chem., 1888. P. 231.
[2] Compt. Rend., 109:479.
[3] Zeitschr. Angew. Chem., 1890. P. 421.
[4] A. H., 10:399 to 444. Chem. Ctr.-Bl., 1890. P. 233.

tion would change the dextrose to alcohol, and the residue would be inactive, while glucose, if it had been used, would leave a dextro-rotatory residue, due to the unchanged starch dextrins present. However, he remarked that all honeys contained a dextrin-like substance, which was more or less readily fermented. Such an unfermentable constituent existed, according to Amthor and Stern[1] and also according to von Raumer.[2] Thus, Sieben's method,which depended upon the total absence of such an unfermentable constituent in pure honeys, was shown to be incorrect. Furthermore, in 1894, Partheil[3] obtained markedly varying results with different fermentation periods. In 1896, Kunnemann and Hilger[4] pointed out the source of error in Sieben's method. He prescribed the use of compressed yeast, which acted more readily on honey dextrins than on starch dextrins, and caused inactivity in the case of the pure honeys which he analyzed. Thus, not knowing that all honeys contained a honey dextrin in variable quantities, and variously affected by the length of the fermentation period, he jumped at the conclusion which he set forth in his method. Beckmann[5] in 1896 added a few

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[1] Zeitschr. Angew. Chem., 1889. P. 576.
[2] Ibid., p. 607.
[3] Apoth. Zeitung., 9:662.
[4] Forschb. ü. Lebensm., etc., 3:329, 330.
[5] Zeitschr. Anal. Chem., 1896. Pp. 263 to 284.

data on the subject of fermentation. Yeast fermented all but 17 percent of pine tree honey dextrin; 27 percent of grape sugar dextrin, precipitated by C_2H_5OH; 40 percent of glucose dextrin when precipitated by C_2H_5OH, and 62 percent when precipitated by CH_3OH. While considering that normal honey dextrins or pine tree honey dextrins were more readily fermented than starch dextrins, he did not consider this reliable as a method of separation.

Another method devised by Sieben[1] was based on the destruction of the levulose by continued boiling with HCl for three hours. The acid was neutralized with NaOH, and the dextrose was determined by Allihn's method.

Finally, Sieben[1] claimed to be able to detect glucose by inverting the cane sugar, neutralizing the acid, and determining the reducing sugars. The filtrate was inverted for a second time with one-tenth of its volume of acid for one hour. Any dextrin present was in this way converted to dextrose, and showed in the reduction of Fehling's solution. More than two mg. of CuO, thus obtained, indicated admixture of glucose. Barth[2] pointed out that even pure honeys sometimes contained sufficient dextrinous matter to give more

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[1] Zeitschr. Anal. Chem., 1885. P. 136.
[2] Pharm. Ctr.-H., 26:87 to 93.

than two mg. of CuO.

In 1890, Haenle[1] became interested in the effect of dialysis on honeys. As a result of his experiments, he stated that all pure honeys became either inactive or levo-rotatory after dialysis, while glucose adulteration became evident in a dextro-rotation. To this statement, Dieterich[2] took exception, claiming from experience that prolonged dialysis caused dextro-rotation in all honeys, the exact point of neutrality being impossible of determination. Each man's followers took up the argument. Sendele[3] and Neuberger[4] upheld Haenle's view, and went so far as to send cleverly adulterated honey samples to several eminent chemists, among whom Haenle was the only one to discover the adulteration. This triumph was cut short by Amthor[5] who returned this sample, after further additions, to Haenle, who pronounced it pure. The invariable dextro-rotation spoken of by Dieterich was explained by Mansfeld as due to the more rapid diffusion of the levulose. Amthor followed up the matter only to discover that after five days' dialysis not only all honey solutions but even dilute glucose solutions became inactive.

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[1] Pharm. Zeitung., 1890. P. 441.
[2] Zeitschr. Nahr. Hyg. Waarenk., 1892. P. 437.
[3] Ibid.. P. 272.
[4] Ibid., 1893. Pp. 163 to 165.
[5] Viertelschr. Chem. Nahr. Genussm., 1893. P. 24.

Although Partheil[1] in 1894 pretended to be able to distinguish, by means of the degree of dextro-rotation, whether the honey was of coniferous origin or adulterated with glucose, the method was discarded as impracticable and inaccurate. Still, von Raumer,[2] while acknowledging the impracticability of direct dialysis suggested the value of dialysis after fermentation, since the honey dextrins dialyzed so much more readily than the starch dextrins. In 1896, Bottler[3] remarked that dialysis proved of value in detecting adulteration only in case impure glucose, that is, glucose containing dextrin, had been added to flower honeys. Beckmann[4] concluded that while glucose dialyzed more slowly, still, it dialyzed just as completely as flower honeys, but that dextrins insoluble in CH_3OH could not be dialyzed.

The latest method of glucose determination was devised by Leach.[5] Assuming that 26.048 gr. of glucose polarized + 175° at ordinary temperatures on the sugar scale, in a 200 mm. tube, and + 163° at 87° C.; these being the average figures for commercial glucose, he calculated the amount of glucose present in a given mixture, from the formula:

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[1] Apoth. Zeitung., 9:662.
[2] Zeitschr. Anal. Chem., 33:397 to 408.
[3] Zeitschr. Nahr. Hyg. Waarenk., 1895. Pp. 69, 70.
[4] Zeitschr. Anal. Chem., 1896. P. 263.
[5] Jour. Amer. Chem. Soc., 25:982 to 987.

$$G = \frac{(a - s)\,100}{175}$$, at ordinary temperatures.

where G = percent glucose; a = direct polarization of the mixture on the Ventzke-Scheibler sugar scale; s = percent cane sugar according to Clerget. Since at 87° C., invert sugar polarizes ± 0 , he divided the invert rotation of the honey at 87°C., by 163, the invert rotation for glucose at 87°C., for the approximate percentage of glucose.

When invert sugar is used to adulterate honeys, its detection is fairly impossible, since invert sugar is the main constituent of pure honeys. In 1894, Deltour[1] suggested that the ratio between dextrose and levulose might prove of some value in distinguishing the invert sugar naturally present in honey from that which was artificial. However, he considered the variable rotation of the levulose with the temperature an obstacle in the operation. Two years later Wiley[2] published his formula for determining the levulose in a honey, or other saccharine product. This formula depended upon this variable rotation of levulose. He discovered that one gram of levulose in one hundred cubic centimeters of solution caused a definite rotation, which

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[1] Rev. Intern. Falsif., 7:182, 183.
[2] Journ. Amer. Chem. Soc., 1896. Pp. 81 to 90.

decreased uniformly with the increase of temperature. When the temperature was raised to 87° C., the levulose rotated just as far to the left as the dextrose did to the right, the result being $\pm 0°$. At 15° C., a normal solution of levulose rotates $-148.6°$ on the sugar scale. The change per degree Centigrade was 0.0357° per gram of levulose. The formula, as it is derived from these data reads:

$$L = \frac{R - R'}{0.0357\ (t - t')}$$

where L = weight of levulose; R = rotation at the higher temperature t; R' = rotation at lower temperature t'; and

$$L' = \frac{L \times 100}{W},$$

where L' = percent of levulose; W = the weight of honey which is made up to 100 ccs. This is usually 26.048 grs. the normal weight for the instrument.

Invert sugars are, however, as a rule, determined according to the method of Allihn or by Defren-O'Sullivan's modification of this method, as they are given by Leach. Both depend upon the reduction of alkaline copper sulphate to Cu_2O, which is converted to CuO and weighed. Tables have been compiled from which the amount of invert sugars, taken as dextrose, may be calculated from the amount of CuO.

Starch, though an extremely crude adulterant, was often tested for either by means of an iodine solution, as given by Krekel[1] in 1888, or microscopically.

Molasses, likewise a very crude adulterant, was determined, according to Krekel, by the large percentage of ash over and above the normal 0.2 percent; also, the large amounts of chlorides, as determined with $AgNO_3$ and the lack of phosphoric acid were marks of distinction between honey ash and molasses ash. Beckmann,[2] in 1896, detected molasses by the addition of lead acetate and methyl alcohol, which revealed as little as ten percent of molasses, because of the heavy precipitate which formed.

Cane sugar, both as an adulterant and as a natural constituent of honeys, is determined in one of two ways: either by the polariscope, using Clerget's formula, or by calculation from the difference between the reducing sugars, before and after inversion. The former method was first used by Lassaigne[3] in 1849. The rotation of the normal solution is determined before and after inversion at the same temperature. Then:

$$\%\ \text{Cane Sugar} = \frac{100\ (a - b)}{144 - \frac{t}{2}}$$

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[1] Pharm. Post.; National Drug., 1888. P. 79.
[2] Zeitschr. Anal. Chem., 1896. Pp. 263 to 284.
[3] Compt. Rend., 28:774, 775.

where a = direct rotation of 26.048 grs., made up to 100 ccs.; b = invert rotation; t = temperature. In 1898, Frühling[1] mentioned a most important fact in sugar analysis, namely, that a honey solution, due to its content of invert sugars, did not give a normal or true reading unless heated, or treated with a drop or two of ammonia, or unless allowed to stand for twenty-four hours. This was called the phenomenon of bi-rotation. In 1902, Tolman[2] stated that the levo-rotation varied in proportion to the amount of HCl used, other things being equal; also that the levo-rotation increased, in the presence of HCl, in proportion to the temperature. He suggested that a correction ought to be made in Clerget's formula for the HCl. Also, he reccommended 20° C., as the proper temperature for the readings.

Lenz,[3] in 1885, recommended that cane sugar be calculated from the reducing sugars before and after inversion. The difference in the amount of reducing sugar multiplied by the factor 0.95 gave the amount of cane sugar, according to Mansfeld.[4] Krekel,[5] in 1888, stated that more than 5 per-

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[1] Zeitschr. Öffent. Chem., 4:174 to 178.
[2] Journ. Amer. Chem. Soc., 1902. Pp. 515 to 524.
[3] Zeitschr. Anal. Chem., 1885.
Chem. Ctr.-Bl., 1885. P. 152.
[4] Zeitschr. Allg. Osterr. Apoth. Ver., 1891. P. 339.
[5] Pharm. Post.; National Dr. 1888. P. 79.

cent of cane sugar indicated an adulteration. The present United States standard allows a maximum of 8 percent. Another method, no longer used after a cane sugar content was considered normal, was due to Hager,[1] in 1885. When H_2SO_4 was added carefully to honey, a cane sugar honey formed a brown contact layer which caused the liquid, when shaken, to turn black and opaque. Honey formed a very slight, if any, layer, and the solution, when shaken, was brown and transparent.

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[1] Chem. Ctr.-Bl., 1885. P. 764.

EXPERIMENTAL

A collection of honeys, which had been obtained by the secretary of the National Bee Keepers' Association from different members of the association, was placed at my disposal and a number of samples were chosen for analysis. This collection had been on exhibition at the St. Louis Exposition, and the samples were not only, in all probability, pure but represented honeys gathered while only one flower was in blossom, as nearly as this could be regulated by the bee-keeper.

The methods followed in analysis were very nearly those given in Leach,- Food Inspection and Analysis, the object being to determine the composition of pure honeys. Inasmuch as the honeys were various in kind and from widely separated localities their comparison may prove of interest. In every case there were determined: the percentage of ash; the percentage of solids; the percentage of cane sugar; the percentage of reducing sugar, and the rotation at an elevated temperature.

In the cane sugar determination half the normal weight, 13.024 grs. of honey was weighed in a tared glass beaker; dissolved in hot water, and clarified by means of a drop or two of lead subacetate and a few cubic centimeters of alumina cream. After cooling, the solution was made up to 100 cubic centimeters in a graduated flask and allowed to stand twenty-four hours. It was then filtered, and a reading was taken at about 20° C., in a 200 mm. tube in a Schmidt and Haensch sugar polariscope. The reading was multiplied by 2 to give the reading for a normal solution. 50 ccs. of this solution, with 5 ccs. of concentrated HCl, were gradually heated up to 68° C., at which temperature all the cane sugar had been inverted. The operation was carried on in a water bath, and so timed that it consumed about fifteen minutes. Care was exercised in preventing a rise in temperature above 70° C., since in such an event any dextrinous matter possibly present would have become inverted. When the solution had cooled down sufficiently a polariscope reading was taken at the same temperature as the first reading, and the cane sugar was calculated by Clerget's formula, already mentioned:

$$\%\ \text{C. S.} = \frac{100\,(a - b)}{144 - \frac{t}{2}}.$$

50 ccs. of the inverted solution were then introduced into a 50 to 55 cc. flask, neutralized with strong KOH, using

phenolphthalein as an indicator and made up to 55 ccs. The solution was then placed in a water jacketed tube, and the temperature was raised by means of a constant temperature apparatus, which allowed a continuous stream of hot water to flow through the jacket of the tube. The temperature was usually raised to between 50° and 70° C., and the reading at 87° C., was calculated from the difference between the readings at the elevated temperature and that at the original temperature, since this supplied the change per degree rise in temperature.

The cane sugar was likewise determined by calculation from the increase in reducing sugars, after inversion, using a modification of Defren-O'Sullivan's method. 30 ccs. $CuSO_4$ solution + 30 ccs. alkaline tartrate solution + 50 ccs. distilled water were introduced into an Erlenmeyer flask and heated in a boiling water-bath for five minutes. Then 25 ccs. of a honey solution, so diluted that about 0.12grs. of sugar was used in each determination, were introduced and heated in the boiling water exactly fifteen minutes. The solution was then filtered through a weighed Gooch platinum crucible containing a layer of asbestos fibre; the process was hastened by means of a suction flask. The Gooch crucible, after sufficient washing with boiling water, was dried in an oven and heated to a dull red heat in the oxidizing part of the Bunsen flame for fifteen minutes. After cooling

it was weighed. The difference in weight was due to CuO, and the percentage of reducing sugars was calculated from Defren's tables as dextrose. The solution was prepared by weighing accurately about 2grs. of honey, making up to 100 ccs., diluting 25 ccs. of the first solution to 100 ccs. and using 25 ccs. of this second solution for a determination. To determine the reducing sugars after inversion 25 ccs. of the first solution were treated with 5 ccs. HCl, inverted by heating gradually to 68° C., neutralized by KOH, using phenophthalein as an indicator, and making up to 100 ccs. 25 ccs. of this inverted solution was used for a second determination of reducing sugars as before. The difference between the reducing sugars before and after inversion was due to cane sugar, and was calculated into cane sugar by multiplying by 0.95.

The total solids in honey were determined indirectly. 25 grs. of honey were dissolved in water and made up to 100 ccs. The specific gravity was determined at 15° C., on the Westphal Balance, and the total solids were computed from a formula:-

$$T.\ S. = 4\ D.\ S.$$

where D = specific gravity, and S = percentage of solids (sugars) as taken from Table VI., in the Appendix of Bulletin, No. 65, of the Bureau of Chemistry, United States Department of Agriculture.

To determine the ash content, 25 ccs. of the solution made up for the specific gravity determination, corresponding to 6.25 grs. of honey, were introduced into a weighed platinum dish, the honey evaporated and charred, and then burned down in a muffle oven. The dish was cooled and weighed. The difference in weight divided by 6.25 gave the percentage of ash in the honey.

On the following pages will be found a compilation of the results obtained. Column B represents the theoretical invert reading at the same temperature as the actual invert reading calculated from the levulose. The levulose is calculated according to Wiley's formula, from invert rotation at ordinary and at elevated temperatures. The levulose multiplied by 2 gives the theoretical amount of invert sugar, and this, in turn, is converted by the factor 0.95 into the equivalent amount of cane sugar. The calculated invert reading of the cane sugar is placed in column B. Column C represents the theoretical invert reading, calculated from the total solids. Assuming that all the solids are invert sugar and cane sugar, the cane sugar is subtracted from the solids to give the invert sugar. This is converted by the factor 0.95 into the equivalent amount of cane sugar, the originally present cane sugar is added, and the invert rotation calculated for the entire amount is

placed in Column C. The figures in column D represent the invert rotation calculated from the total reducing sugar; that is, the reducing sugar after inversion. This total invert sugar is calculated to cane sugar, as before, and the theoretical invert reading placed in column D. B - A, C - A, and D - A represent the differences between the actual invert rotation and the calculated invert rotations.

The results, as described above, were not given for Numbers 12 and 29 because of unsatisfactory high temperature readings. Number 71 is an old honey, with an abnormally high cane sugar content, and was presumably not gathered under the precautions already stated. Number 53 was considered suspicious because of its syrupy consistency, which resembled glucose more than honey. In addition to an abnormal cane sugar content, it showed an especially high dextro-rotation at 87° C., and gave a very decided precipitate with lead subacetate.

Several of the honeys which were especially dextro-rotatory at 87° C., were treated with ethyl and with methyl alcohol, according to Beckmann. The honeys so tested were Numbers 1, 24, and 53. In each case, a brownish, flocculent precipitate, presumably honey dextrin, was formed, which was most voluminous in the case of Number 53.

DATA

Number of Specimen	Kind	Specific Gravity at 15° C.	Solids	Moisture	Ash	Temperature of Polariscope Readings
			%	%	%	°C.
1	White Clover,	1.0787	81.89	18.11	0.140	18.2
9	Raspberry,	1.0795	82.70	17.30	0.055	16.4
10	Alfalfa,	1.0794	82.63	17.37	0.057	20.8
11	Sage,	1.0798	83.03	16.97	0.080	24.9
12	Cotton,	1.0741	77.07	22.93	0.150	22.6
13	Melon,	1.0774	80.50	19.50	0.300	20.8
14	White Clover,	1.0792	82.45	17.55	0.090	22.5
19	Clover,	1.0788	81.98	18.02	0.142	25.0
24	Fall Flowers,	1.0787	81.89	18.11	0.401	16.0
28	Basswood,	1.0798	83.03	16.97	0.203	22.5
29	Orange Bloom,	1.0778	80.94	19.06	0.087	22.5
30	Buckwheat,	1.0780	81.17	18.83	0.128	21.3
31	Laurel,	1.0778	80.94	19.06	0.320	26.2
32	Wild Buckwheat,	1.0770	80.82	19.18	0.107	23.2
33	Alsike Clover,	1.0791	82.33	17.67	0.059	24.5
36	Mangrove,	1.0768	78.89	21.11	0.191	21.0
47	Mexico,	1.0763	79.38	20.62	0.224	20.7
49	Saw Palmetto,	1.0776	80.71	19.29	0.310	16.5
50	Wild Buckwheat,	1.0790	82.21	17.79	0.160	16.4
51	Wild Buckwheat,	1.0783	81.52	18.48	0.076	21.6
52	White Clover,	1.0827	80.99	19.01	0.060	18.8
53	White Oaks,	1.0832	86.65	13.35	0.922	22.5
62	Clover,	1.0820	85.34	14.66	0.080	19.5
71	Dandelion,	1.0806	83.85	16.15	0.104	22.0
90	White Clover,	1.0778	80.94	19.06	0.0605	25.2

DATA

Direct Rotation	Invert Rotation	Reducing Sugars Direct	Reducing Sugars Invert	Cane Sugar Calculated from Rotation	Cane Sugar Calculated from Reducing Sugar	Localities
		%	%	%	%	
-12.7	-15.62	78.86	80.66	2.38	1.71	Oregon.
-20.6	-24.20	82.22	84.98	2.64	2.62	Maine.
-18.4	-22.44	84.61	87.28	2.64	2.54	Kansas.
-18.0	-22.66	83.23	86.51	3.54	3.11	California.
-22.0	-23.74	80.43	81.62	1.32	1.13	South Carolina
-14.0	-17.16	78.37	80.37	2.36	1.90	Illinois.
-12.0	-14.30	84.31	85.93	1.73	1.53	Michigan.
-13.0	-15.40	83.18	84.78	1.82	1.52	Michigan.
- 8.8	-13.20	74.66	78.02	3.23	3.19	Minnesota.
-16.0	-19.14	81.17	83.16	2.36	1.89	Mississippi.
-25.4	-29.48	80.06	82.73	3.07	2.61	California.
-16.9	-18.26	81.57	82.94	1.01	1.30	Michigan.
-17.0	-18.70	82.02	83.75	1.29	1.64	Florida.
-20.7	-23.76	81.01	82.96	2.31	1.91	California.
-12.8	-15.62	82.22	84.23	2.14	1.90	Wisconsin
-25.5	-26.62	80.54	83.60	0.83	0.218	Florida.
-12.8	-15.62	75.00	76.94	2.11	1.84	Mexico.
-17.6	-20.90	77.50	80.26	2.43	2.62	Florida.
-17.6	-20.24	78.75	80.40	1.94	1.56	California.
-16.0	-18.48	82.77	84.83	1.86	1.95	Wisconsin.
-13.8	-17.38	80.67	83.12	2.65	2.61	New York.
+17.8	+ 6.60	67.02	76.64	8.43	9.13	Arkansas.
-11.2	-13.75	83.19	85.51	1.89	2.20	Wisconsin.
- 3.6	-18.48	76.59	87.80	11.18	10.65	Wisconsin.
-11.6	-14.74	80.54	83.60	2.38	2.90	Wisconsin.

DATA (Continued)

Number of Specimen	Kind	Invert Rotation Found A	Invert Rotation Calculated from Levulose B	Invert Rotation Calculated from Solids C	Invert Rotation Calculated from Total Reducing Sugars D
1	White Clover,	-15.62	-27.31	-27.18	-26.74
9	Raspberry,	-24.20	-29.46	-28.24	-28.98
10	Alfalfa,	-22.44	-21.96	-25.80	-28.29
11	Sage,	-22.66	-26.77	-23.74	-25.84
12	Cotton,				
13	Melon,	-17.16	-25.54	-25.73	-25.65
14	White Clover,	-14.30	-23.72	-25.41	-26.73
19	Clover,	-15.40	-24.35	-24.55	-25.37
24	Fall Flowers,	-13.20	-27.63	-28.06	-26.68
28	Basswood,	-19.14	-26.69	-25.87	-25.87
29	Orange Bloom,				
30	Buckwheat,	-18.26	-23.31	-25.73	-26.13
31	Laurel,	-18.70	-23.30	-23.77	-24.68
32	Wild Buckwheat,	-23.76	-27.90	-24.90	-25.92
33	Alsike Clover,	-15.62	-24.91	-24.86	-25.33
36	Mangrove,	-26.62	-25.01	-24.84	-26.64
47	Mexico,	-15.62	-25.40	-24.70	-24.59
49	Saw Palmetto,	-20.90	-29.47	-27.45	-27.25
50	Wild Buckwheat,	-20.24	-28.24	-27.88	-27.22
51	Wild Buckwheat,	-18.48	-24.79	-25.73	-26.76
52	White Clover,	-17.38	-25.50	-26.66	-27.42
53	White Oaks,	+ 6.60	-24.08	-27.09	-23.84
62	Clover,	-13.75	-27.38	-27.83	-27.86
71	Dandelion,	-18.348	-26.86	-27.07	-28.15
90	White Clover,	-14.74	-24.33	-24.18	-24.93

DATA (Continued)

A - B	B - C	C - D	Invert Rotation at 87° C.	Localities
11.69	11.56	11.12	+ 11.26	Oregon.
5.26	4.04	4.78	+ 4.26	Maine.
4.52	3.36	5.85	+ 3.58	Kansas.
4.11	1.08	3.18	+ 3.15.	California.
				South Carolina.
8.38	8.57	8.49	+ 7.46	Illinois.
9.42	11.37	12.43	+ 9.90	Michigan.
8.95	9.15	9.97	+ 8.49	Michigan.
14.43	14.86	13.48	+ 13.025	Minnesota.
7.55	6.73	6.73	+ 8.68	Mississippi.
				California.
5.05	7.37	7.87	+ 7.79	Michigan.
4.60	5.07	5.98	+ 5.70	Florida.
4.14	1.14	2.16	+ 3.90	California.
9.29	9.24	9.71	+ 8.38	Wisconsin.
- 1.61	- 1.78	0.02	- 2.49	Florida.
9.78	9.08	8.97	+ 9.21	Mexico.
8.57	6.55	6.35	+ 9.43	Florida.
8.00	7.64	6.98	+ 7.03	California.
6.31	7.25	8.27	+ 5.26	Wisconsin.
8.12	9.28	10.04	+ 7.17	New York.
30.68	33,69	30.44	+ 29.82	Arkansas.
13.63	14.08	14.11	+ 12.60	Wisconsin.
8.51	8.59	9.67	+ 9.64	Wisconsin.
9.59	9.44	10.19	+ 8.68	Wisconsin.

CONCLUSIONS

In the experimental work with honeys, it was observed that the polariscopic method for determining cane sugar gave more satisfactory results than the method of calculating from the invert sugar before and after inversion. The duplicates agreed more closely, pointing to greater accuracy, and the rapidity of the process recommended it. On the other hand, the results calculated from the reducing sugars were invariably lower than the polariscopic results, pointing to losses inherent in the process, because of its length and complicated nature.

Contrary to statements made by Leach and others, the data show that dextro-rotation at 87° C., is the rule rather than the exception. Since invert sugar polarizes ±0 at 87° C., the dextro-rotation must be caused by some other substance normally present in honey. The presence of a dextro-rotatory substance, other than the dextrose of the invert sugar; becomes apparent in the low levo-rotation actually found at ordinary temperatures, given in column A, as com-

pared with the calculated levo-rotations found in columns B, C, and D, which latter are based on the supposition that the only optically active substance present in the honey is invert sugar. The lowering of the levo-rotation must, therefore, be due to the presence of a dextro-rotatory constituent, and at 87° C., where the invert sugar becomes inactive, the dextro-rotation appears as such. Note the close analogy between the dextro-rotation at 87° C., and the difference between the actual and the calculated levo-rotations as expressed in columns B - A, C - A, and D - A. In the majority of cases, the dextro-rotation at 87° is slightly lower than the figures in columns B - A, C - A, and D - A, as might be expected, since even glucose gives lower readings at 87° C., than at ordinary temperatures, due to the difference in expansion of the solution and the glass of the observation tube. The results prove that a dextro-rotation not exceeding 15° at 87° C., cannot be considered as conclusive evidence of the adulteration of a sample of honey with glucose.

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